

We Claim:

1. A non-naturally occurring mutant human hemoglobin wherein the asparagine residue at position 108 of the  $\beta$ -chains (SEQ ID NO: 5) is replaced by a glutamine residue.

5 2. The hemoglobin of Claim 1 possessing low oxygen affinity as compared to normal human adult hemoglobin.

3. The hemoglobin of Claim 2 further possessing high cooperativity in oxygen binding comparable to normal human adult hemoglobin.

10 4. The hemoglobin of Claim 3 further possessing increased stability against autoxidation.

5. The hemoglobin of Claim 1 which is produced recombinantly.

6. rHb ( $\beta$ N108Q) (SEQ ID NO: 5).

15 7. An artificial mutant hemoglobin which in a cell-free environment has oxygen binding properties comparable to those of human normal adult hemoglobin in red blood cells wherein said hemoglobin contains a mutation such that the asparagine residue at position 108 of the  $\beta$ -chains is glutamine (SEQ ID NO: 5).

8. The hemoglobin of Claim 7 which is produced recombinantly.

20 9. A non-toxic pharmaceutical composition comprising a non-naturally occurring mutant hemoglobin wherein the asparagine residue at position 108 of the  $\beta$ -chains is replaced by a glutamine residue (SEQ ID NO: 5) in a pharmaceutically acceptable carrier.

10. The composition of Claim 9 wherein said hemoglobin in a cell-free environment has oxygen binding properties lower than those of human normal adult hemoglobin.

11. The composition of Claim 10 wherein said hemoglobin is  
5 rHb ( $\beta$ N108Q) (SEQ ID NO: 5).

12. Plasmid pHE7009.

13. A non-naturally occurring low oxygen affinity mutant hemoglobin with increased stability against autoxidation that has oxygen binding properties comparable to those of human normal adult hemoglobin in the presence of the allosteric  
10 effector 2,3-bisphosphoglycerate, wherein the asparagine residue at position 108 of each of the  $\beta$ -chains is replaced by a glutamine residue (SEQ ID NO:5).

14. A non-naturally occurring mutant human hemoglobin wherein said hemoglobin contains a mutation of the asparagine residue at position 108 of the  $\beta$ -chains (SEQ ID NO: 5) possessing oxygen-binding properties of oxygen affinity as  
15 measured by  $P_{50}$  and cooperativity as measured by the Hill coefficient ( $n_{\max}$ ) and similar to those of Hb A in the presence of the allosteric effector 2,3-bisphosphoglycerate as follows:  $P_{50}$  about 17.4 mm Hg,  $n_{\max}$  about 3.1 in 0.1 M sodium phosphate at pH 7.4 and 29°C.

15. A method of producing artificial hemoglobin, comprising:

introducing an expression plasmid which contains a DNA coding sequence for human hemoglobin wherein the asparagine residue at position 108 of the  $\beta$ -chains is replaced by glutamine residue (SEQ ID NO:5) into a suitable host other than

5 an erythrocyte and growing the transformed cells;

expressing said DNA to produce said artificial hemoglobin; and  
recovering and purifying said hemoglobin.

16. The method of Claim 15, wherein said host cells are E. coli.

17. The method of Claim 16, wherein said expression plasmid is

10 pHE7009

18. A method of treating a human subject, comprising administering to said subject a nontoxic composition comprising an artificial mutant hemoglobin, wherein said artificial mutant hemoglobin is rHb ( $\beta$ N108Q) (SEQ ID NO: 5).

15 19. rHb ( $\beta$ N108Q) (SEQ ID NO: 5) derived from cells transformed with pHE7009.

20. A non-naturally occurring mutant human hemoglobin wherein the leucine residue at position 105 of the  $\beta$ -chains (SEQ ID NO: 7) is replaced by a tryptophan residue.

21. The hemoglobin of Claim 20 possessing low oxygen affinity as  
20 compared to normal human adult hemoglobin.

22. The hemoglobin of Claim 21 further possessing high cooperativity in oxygen binding comparable to normal human adult hemoglobin.

23. The hemoglobin of Claim 20 which is produced recombinantly.

24. rHb ( $\beta$ L105W) (SEQ ID NO:7).

25. An artificial mutant hemoglobin which in a cell-free environment has oxygen binding properties comparable to those of human normal adult hemoglobin in red blood cells wherein said hemoglobin contains a mutation such that the leucine residue at position 105 of the  $\beta$ -chains is tryptophan (SEQ ID NO: 7).

26. The hemoglobin of Claim 25 which is produced recombinantly.

27. A non-toxic pharmaceutical composition comprising a non-naturally occurring mutant hemoglobin wherein the leucine residue at position 105 of the  $\beta$ -chains is replaced by a tryptophan residue (SEQ ID NO:7) in a pharmaceutically acceptable carrier.

28. The composition of Claim 27 wherein said hemoglobin in a cell-free environment has oxygen binding properties lower than those of human normal adult hemoglobin.

29. The composition of Claim 28 wherein said hemoglobin is rHb ( $\beta$ L105W) (SEQ ID NO: 7).

30. Plasmid pHE7004.

31. A non-naturally occurring low oxygen affinity mutant hemoglobin that has oxygen binding properties comparable to those of human normal adult hemoglobin in the presence of the allosteric effector 2,3-bisphosphoglycerate, wherein the leucine residue at position 105 of each of the  $\beta$ -chains is replaced by a tryptophan residue (SEQ ID NO: 7).

32. A non-naturally occurring mutant human hemoglobin wherein said hemoglobin contains a mutation of the leucine residue at position 105 of the  $\beta$ -chains (SEQ ID NO: 7) possessing oxygen-binding properties of oxygen affinity as measured by  $P_{50}$  and cooperativity as measured by the Hill coefficient ( $n_{max}$ ) and similar  
5 to those of Hb A in the presence of the allosteric effector 2,3-bisphosphoglycerate as follows:  $P_{50}$  about 28.2 mm Hg,  $n_{max}$  about 2.6 in 0.1 M sodium phosphate at pH 7.4 and 29°C.

33. A method of producing artificial hemoglobin, comprising:  
introducing an expression plasmid which contains a DNA coding  
10 sequence for human hemoglobin wherein the leucine residue at position 105 of the  $\beta$ -chains is replaced by tryptophan residue (SEQ ID NO: 7) into a suitable host other than an erythrocyte and growing the transformed cells;  
expressing said DNA to produce said artificial hemoglobin; and  
recovering and purifying said hemoglobin.

15 34. The method of Claim 33, wherein said host cells are E. coli.

35. The method of Claim 34, wherein said expression plasmid is pHE7004.

36. A method of treating a human subject, comprising administering to said subject a nontoxic composition comprising an artificial mutant hemoglobin,  
20 wherein said artificial mutant hemoglobin is rHb ( $\beta$ L105W) (SEQ ID NO: 7).

37. rHb ( $\beta$ L105W) derived from cells transformed with pHE7004.